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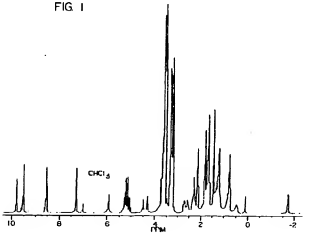
(54) Pyropheophorbides and their use in photodynamic therapy

(57) Pyropheophorbidas compounds ara injected into a host and accumulate in tumor tissue to a higher degree than surrounding normal tissues. When the pyropheophorbida compounds are exposed to a particular wavelenght of light the compounds become cytotoxic and destroy the tumor or diseased tissue without causing irreversible normal tissues damage. The phyropheophorbide compounds have shown improved results as compared to drugs currently usad in photodynamic therapy. Further, they absorb light further in the red, optimizing tissue penetration and are retained in the skin for short time periods relative to other drugs used in photodynamic therapy.

FIG. 1



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Description

Cross-References

[0001] This application is a continuation-in-part of our earlier filed application Serial No. 07/597,786 filed October 15, 1990 which is a continuation of application Serial No. 07/221,804 filed July 20, 1988 which is now U.S. Patent 5,002,962 issued March 26, 1991 both of which are incorporated herein by reference and to which we claim priority under 35 USC §120.

FIELD OF THE INVENTION

[0002] This invention relates generally to photosensitive therapeutic compounds and photodynamic therapy (PDT). More particularly, the invention relates to pyropheophorbides, formulations that contain such and their use in the treatment of cancer.

BACKGROUND OF THE INVENTION

[0003] As described in U.S. Patent 5,002,962, porphyrin related compounds accumulate at higher concentrations in tumor tissue as compared to normal tissue, and that irradiation of these compounds using light of the proper wavelength results in an energized form which, upon decay, results in cytotoxicity. It is believed that excitation of the porphyrin or related materiel results in the formation of singlet oxygen which is in fact the toxic agent. However, the compounds administered apperently do not degrade in this process.

[0004] Literature relating to the use of "hematoporphyrin derivative" (HPD) describes this process utilizing a preparation obtained when hematoporphyrin dichloride is treated using the procedure of Lipson, R.L., et al, J.Nationel Cancer Inst (1961) 26:1-8. More recently, it has been shown that if this hematoporphyrin derivative is treated at a suitable pH, aggregation occurs and the active material in the mixture can be prepared in crude form as a size segregated aggregate (see, for example, U.S. Patent 4,649,151, incorporated herein by reference). This preparation is commercially available under the trademark Photofrin.

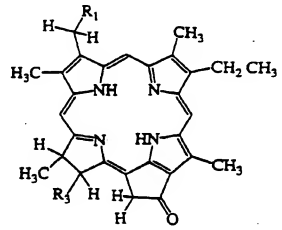
[0005] The preparation marketed as the Photofrin composition is e mixture. The mixture contains porphyrins joined by ether linkages (Dougherty, T.J., et al, Adv Exp Med Bio (1983) 160:3-13), and more recently, Kessel, D., et al Photochem Photobiol (1987) 46:463-568, has shown that ester linked porphyrins are contained in this mixture as well. Scourides, P.A.; et el, Cancer Res (1987) 47:3439-3445 heve synthesized an oligomeric mixture of ether linked porphyrins starting from hematoporphyrin dimethyl esters. The mixture was active in PDT, but was as complex a mixture as the Photofrin preparation. Dimers of hematoporphyrin joined by ester linkeges have also been prepared by Pandey, R.K., et el, Cancer Res (in press) and the dimers prepered were shown to be absent from the mixture in the Photofrin composition as well as inactive in en in vitro assey.

[0006] Thus, it is known in the art that some elements of a mixture prepared when HPD is aggregated and segregated into higher molecular weight components ere ective in photodynamic therepy. Earlier, the present inventors prepared single compound compositions useful in PDT as disclosed in U.S. Patent 5,002,962. The purified and defined compositions disclosed in U.S. Petent 5,002,962 are useful in photodynamic therapy as are compounds and methods disclosed in U.S. Patents 4,920,143 and 4,883,790.

SUMMARY OF THE INVENTION

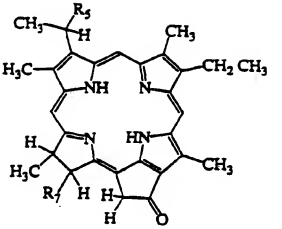
[0007] Pyropheophorbide compounds and pharmaceutical compositions containing such compounds can be used in methods of photodynamic therapy. The pyropheophorbides are encompassed by the following general structurel formula I or II.

I



wherein R_1 is CH_2OR_2 where R_2 is a primary or secondary alkyl containing 1 to 20 carbons; and R_3 is $-CO_2R_4$ where R_4 is H or an alkyl containing 1 to 20 carbons. Other compounds of the invention are encompassed by formula II as follows:

II



wherein R_5 is $-OR_6$ where R_6 is a primary or secondary alkyl containing 1 to 20 carbons and R_7 is $-CO_2R_8$ where R_8 is H or an alkyl containing 1 to 20 carbons. Particularly preferred compounds are where R_5 is -O-hexyl and R_7 is $-CO_2H$ or $-CO_2CH_3$. The pyropheophorbides of the invention are combined with excipients to provide pharmaceutically acceptable formulations suitable for use in photodynamic therapy.

[0008] The invention also includes methods of synthesizing compounds of formula I and II.

[0009] The invention includes injectable pharmaceutical compositions containing the pyropheophorbide compounds of the invention as active ingredients and to methods of conducting photodynamic therapy using the compounds and compositions of the invention.

[0010] The invention also includes the pyropheophorbide compounds of the invention conjugated to a ligand which is capable of binding a specific receptor such as a cellular receptor, or an antibody which is capable of binding to a particular antigen and to compositions containing these conjugates and methods of conducting photodynamic therapy using the conjugates and their compositions.

[0011] A primary object of the invention is to provide pyropheophorbide compounds, pharmaceutical compositions

containing such compounds and method of treatment carried out using such compounds in a photodynamic therapy.

[0012] Other objects are to provide methods of treating humans with tumor cells which cells replicate abnormally fast, treating atherosclerosis or inactivating bacteria or virus infections.

[0013] A feature of the present invention is that the pyropheophorbide compounds of the invention absorb light further into the red portion of the spectrum as compared with conventional compounds used in photodynamic therapy.

[0014] An advantage of the present invention is that the pyropheophorbide compounds and pharmaceutical compositions of the invention optimize tissue penetration and are retained in the skin for relatively short periods of time as compared with other compounds used in photodynamic therapy.

[0015] Another advantage of the present invention is that the pyropheophorbide compounds of the invention have a greater toxicity with respect to tumor cells and diseased tissue as compared with the toxicity of conventional compounds used in photodynamic therapy.

[0016] Another advantage of the invention is that the pyropheophorbides can be synthesized as free acids (e.g. in formula I or II when R_6 or R_7 are $-CO_2H$) allowing ease in formulation without the need for liposomes or detergents.

[0017] Another advantage of the invention is the pyropheophorbide of the invention are active at very low doses of injected material as compared to conventional photosensitizers used in photodynamic therapy.

[0018] These and other objects, advantages and features of the present invention will become apparent to those persons skilled in the art upon reading the details of the structure, synthesis and usage as more fully set forth below, reference being made to the accompanying structural formels forming a part herein wherein like symbols refer to like molecular moieties throughout.

Brief Description of the Drawings

[0019] Figure 1 is a FAB mass spectrum of the compound of formula II(a).

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS OF THE INVENTION

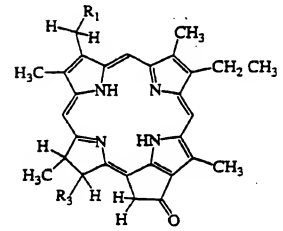
[0020] Before the present pyropheophorbide compounds, pharmaceutical compositions, methods of synthesizing and using such compounds are disclosed, it is to be understood that this invention is not limited to the particular compounds, compositions, methods of use or synthesis as described as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting since the scope of the present invention will be limited only by the appended claims.

[0021] It is to be noted that as used in this specification and the appended claims, the singular forms "a", "and" and "the" include plural reference unless the context clearly dictates otherwise thus, for example, reference to "a pyropheophorbide" includes mixtures of such pyropheophorbides, reference to "an antibody" includes mixtures of such antibodies and reference to "the method of treatment" includes reference to like methods which will become apperent to those skilled in the art upon reading this disclosure.

[0022] Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of photodynamic therapy. Although any methods and materials similar or equivalent to those described herein may be used in the practice or testing of the present invention, attempts have been made to describe preferred methods and materials below.

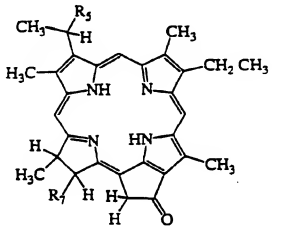
[0023] The essence of the invention is the disclosure of novel compounds and pharmaceutical compositions containing such compounds which have been found to be highly effective in the treatment of cancer when used in connection with a photodynamic therapy. More specifically, the compounds are pyropheophorbide compounds which are encompassed by the following general structural formulae I and II.

I



wherein R_1 is CH_2OR_2 where R_2 is a primary or secondary alkyl containing 1 to 20 (preferably 5-20) carbons; and R_3 is $-CO_2R_4$ where R_4 is H or an alkyl containing 1 to 20 carbons. Preferred compounds are when R_1 is $-CH_2$-O-hexyl and R_3 is $-CO_2CH_3$ or $-CO_2H$. Other compounds of the invention are encompassed by formula II as follows:

II

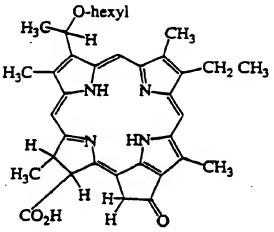


wherein R_5 is $-OR_6$ where R_6 is a primary or secondary alkyl containing 1 to 20 (preferably 5-20) carbons and R_7 is $-CO_2R_8$ where R_8 is H or an alkyl containing 1 to 20 carbons. Particularly preferred compounds are where R_5 is -O-hexyl and R_7 is $-CO_2H$ or $-CO_2CH_3$.

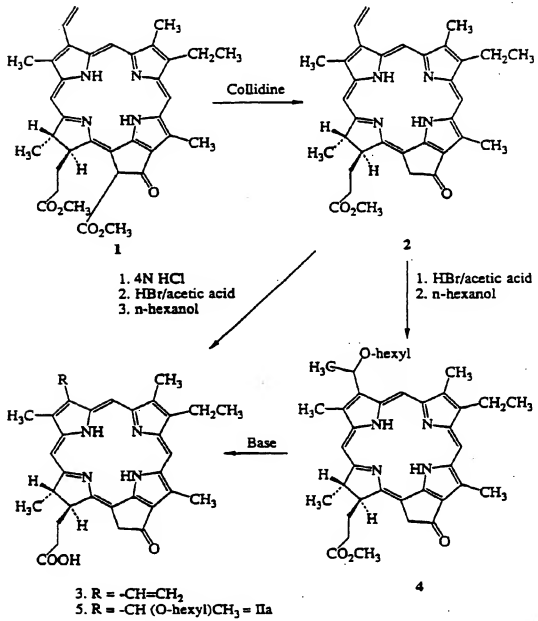
[0024] The pyropheophorbide compounds of structural formulae I and II can be formulated into pharmaceutical compositions and administered to patients in therapeutically effective amounts in order to treat cancer.

[0025] Although the invention encompasses all of the compounds of structural formulae I and II it has been found that the compound of structural formula IIa is particularly effective in the treatment of cancer when used in connection with photodynamic therapy. Structural formula IIa is put forth below:

IIa



[0026] A generalized reaction scheme for the synthesis of the compound of structural formula IIa is put forth below:



Scheme 1

Starting Materials

[0027] The starting material for preparation of the red light-absorbing compounds is methyl pheophorbide-a, which

is isolated from Spirulina destridratada by the method of Smith and Goff (D. Goff, Ph.D. Thesis, Univ. of Calif., Davis, CA 95616, 1984 incorporated herein by reference). Briefly, 500 gm dried Spirulina was slurried in a large volume of acetone and then liquid nitrogen was added to form a frozen slush. The slush was transferred to a 3-necked, 5-liter round bottom flask and heated to reflux under nitrogen with stirring for 2 hours. The mixture was filtered through Whetman papar on a Buchner funnel with extensive acetone washing. The extraction and filtration process was repeatad 2 more times; all green color could not be removed from the solid.

[0028] The green filtrate was evaporated and purified by flash chromatography on Grade V neutral Alumina, eluting first with n-hexane to remove a fast running yellow band and then with dichloromethane to obtain the major blue/gray peak containing pheophytin-a. Treatment of pheophytin-a with 500 ml sulfuric acid in methanol for 12 hours at room temperature in the dark under nitrogen was followed by dilution with dichloromethane. The reaction mixture was rinsed with watar and then 10% aqueous sodium bicarbonata and tha organic layer was dried, avaporated, and the residua recrystallized from dichloromethane/methanol to obtain 1.8 gm methyl pheophorbide-a. Methyl pheophorbide-a appears to be inactive in the In vivo tumoricidal activity assay when injected at a dose of 5 mg/kg.

Conjugates and Labeled Pyropheophorbides

[0029] In addition to using compositions which consist essentially of the above-defined compounds or preparations as active ingredient, it is possible to use derivetized forms in order to provide e specific targeting mechenism. Commonly used target-specific components include monoclonal antibodies and ligands which bind to a celiular receptor. The compositions can also be conveniently labeled.

[0030] The target-specific component can then be, for exemple, an immunoglobulin or portion thereof or e ligend specific for a particular receptor. Tha immunoglobulin component can be eny of a veriety of materials. It may ba darived from polyclonal or monoclonal antibody preparations and may contain whole entibodies or immunologically reactive fragments of these antibodies such as $F(ab')_2$, FAB, or FAB' fragments. Use of such immunologically reactive fragments as substitutes for whola antibodias is wall known in the art. See, for axampla, Spiegelberg, H.L., in "Immunoassays in the Clinical Laboratory" (1978) 3:1-23 incorporated herein by reference.

[0031] Polyclonal anti-sers are prepared in conventional ways by injecting a suitable mammal with antigen to which antibody is dasired, assaying the antibody level in serum against tha antigan, and preparing anti-sera whan tha titers ara high. Monoclonal antibody preparations may also be prepared conventionally such as by the method of Koehler and Milstein using peripheral blood lymphocytes or spleen cells from immunized animals and immortalizing these cells either by viral infection, by fusion with myalomas, or by other conventional procedures, and screening for production of the desired entibodies by isolated colonies. Formation of the fragments from either monoclonal or polyclonal preparations is effected by convantional means as described by Spiegelberg, H.L., supra.

[0032] Particularly useful antibodies include the monoclonal antibody preparation CAMAL1 which can be prepared as described by Malcolm, A., et al, Ex Hemetol (1984) 12:539-547; polyclonel or monoclonel preperetions of enti-M1 antibody as described by Naw, D., et al, J Immunol (1983) 130:1473-1477 (supra) and B16G antibody which is preparad as described by Maier, T., et al, J Immunol (1983) 131:1843; Steele, J.K., et al, Cell Immunol (1984) 90:303 all of which publications ere incorporated herein by reference.

[0033] Tha foregoing list is exemplary and certainly not limiting; once the targat tissua is known, antibody specific for this tissue may ba preparad by conventional means. Therefore the invention is applicable to effecting toxicity against any desired target.

[0034] Tha ligand specific for raceptor rafars to a moiety which binds e receptor at call surfaces, and thus contains contours and charge patterns which are complementary to those of the receptor. It is well understood that a wide variety of cell types have specific receptors designed to bind hormones, growth factors, or neurotransmitters. However, while these embodiments of ligands specific for receptor are known and understood, the phrase "ligand specific for receptor, " as used herein, refers to any substance, natural or synthetic, which binds specifically to a receptor.

[0035] Examples of such ligands include the steroid hormones, such as progesterone, estrogens, androgens, and the adrenal cortical hormones; growth factors, such as epidermal growth factor, nerve growth factor, fibroblast growth factor, and so forth; other protein hormones, such as human growth hormone, parathyroid hormone, and so forth; and neurotransmitters, such as acetylcholine, serotonin, and dopamine. Any analog of these substances which succeeds in binding to the receptor is also included.

[0036] The conjugation of the target-cell-specific component to the compounds of the invention can be effected by any convanient maans. For proteins, such ig and certain receptor ligands, a diract covalant bond batwaen thasa moieties may be effacted, for example, using a dehydreting agent such as a carbodiimide. A particularly preferred method of covalently binding the compounds of the invention to the immunoglobuiln moiety is treatment with 1-ethyl-3-(3-dimathylamino propyl) carbodiimida (EDCI) in the presence of a reaction madium consisting essentially of dimethyl sulfoxide (DMSO).

[0037] Of course, other dehydreting agents such as dicyclohexylcarbodilmide or diethylcarbodilmide could also be

used as well as conventional aqueous and partially aqueous media.

[0038] Nonprotein receptor ligands can be conjugated to be dimers and trimers according to their relevant functional groups by means known in the art.

[0039] The active moieties of the conjugate may also be conjugated through linker compounds which are bifunctional, and are capable of covalently binding each of the two active components. A large variety of these linkers is commercially available, and a typical list would include those found, for example, in the catalog of the Pierce Chemical Co. These linkers are either homo- or heterobifunctional moieties and include functionalities capable of forming disulfides, amides, hydrazones, and a wide variety of other linkages.

[0040] Other linkers include polymers such as polyamines, polyethers, polyamine alcohols, derivatized to the components by means of ketones, acids, aldehydes, isocyanates, or a variety of other groups.

[0041] The techniques employed in conjugating the active moieties of the conjugate to the target-specific component include any standard means and the method for conjugation does not form part of the invention. Therefore, any effective technique known in the art to produce such conjugates falls within the scope of the invention, and the linker moiety is accordingly broadly defined only as being either a covalent bond or any linker moiety available in the art or derivable therefrom using standard techniques.

[0042] The compounds of the invention per se or the conjugates may be further derivalized to a compound or ion which labels the drug. A wide variety of labeling moieties can be used, including radioisotopes and fluorescent labels. Radioisotope labeling is preferred, as it can be readily detected in vivo.

[0043] The compounds which are alone or are conjugates with a specific binding substance can be labeled with radioisotopes by coordination of a suitable radioactive cation in the porphyrin system. Useful cations include technetium and Indium. In the conjugates, the specific binding substances can also be linked to label.

Administration and Use

[0044] In general, the pyropheophorbide compounds of the invention are administered to a host such as a human suffering from cancer in therapeutically effective amounts by any suitable means such as injection which may be IV or IM or may be administered transdermally. The pyropheophorbide compounds of the invention accumulate in tumor cells to a much higher degree than they accumulate in surrounding normal tissues. After being provided with sufficient time so as to accumulate in the tumor tissue, the pyropheophorbide compounds are exposed to a particular wavelength of light which causes the compounds to become cytotoxic, thus destroying the tumor or diseased tissue which the pyropheophorbide compounds have accumulated in. This is accomplished without causing irreversible damage to surrounding normal tissues wherein there has not been an accumulation of the pyropheophorbide compounds.

[0045] The compounds and their conjugates with target-specific substances of the invention are useful, in general, in the manner known in the art for hematoporphyrin derivative and for Photofrin II compositions. These compositions are useful in sensitizing neoplastic cells or other abnormal tissue to destruction by irradiation using visible light -- upon photoactivation, the compositions have no direct effect, nor are they entered into any biological event; however the energy of photoactivation is believed to be transferred to endogenous oxygen to convert it to singlet oxygen. This singlet oxygen is thought to be responsible for the cytotoxic effect. In addition, the photoactivated forms of porphyrin fluoresce which fluoresce can aid in localizing the tumor. Thus, the dimer and trimer compounds of the invention are not consumed or altered in exerting their biological effects.

[0046] Typical indications, known in the art, include destruction of tumor tissue in solid tumors, dissolution of plaques in blood vessels (see, e.g., U.S. patent 4,512,762); treatment of topical conditions such as acne, athlete's foot, warts, papilloma, and psoriasis and treatment of biological products (such as blood for transfusion) for infectious agents, since the presence of a membrane in such agents promotes the accumulation of the drug. Other uses include treating humans suffering from atherosclerosis and inactivating bacaterial or viral infections.

[0047] The compositions are formulated into pharmaceutical compositions for administration to the subject or applied to an in vitro target using techniques known in the art generally. A summary of such pharmaceutical compositions may be found, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania, latest edition. The compositions, labeled or unlabeled, can be administered systemically, in particular by injection, or can be used topically.

[0048] Injection may be intravenous, subcutaneous, intramuscular, or even intraperitoneal. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid form suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol and the like. Of course, these compositions may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and so forth.

[0049] Systemic administration can also be implemented through implantation of a slow release or sustained release system, by suppository, or, if properly formulated, orally. Formulations for these modes of administration are well known in the art, and a summary of such methods may be found, for example, in Remington's Pharmaceutical Sciences

(supra).

[0050] If the treatment is to be localized, such as for the treatment of superficial tumors or skin disorders, the compositions may be topically administered using standard topical compositions involving lotions, suspensions, or pastes.

[0051] The quantity of compound to be administered depends on the choice of active ingredient, the condition to be treated, the mode of administration, the individual subject, and the judgment of the practitioner. Depending on the specificity of the preparation, smaller or larger doses may be needed. For compositions which are highly specific to target tissue, such as those which comprise conjugates with a highly specific monoclonal immunoglobulin preparation or specific receptor ligand, dosages in the range of 0.05-1 mg/kg are suggested. For compositions which are less specific to the target tissue, larger doses, up to 1-10 mg/kg may be needed. The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large and considerable excursions from these recommended values are expected. Further, because of slight solubility in water, certain compounds of the invention may be administered directly in saline or 5% glucose solution, thus avoiding the complications of detergents or other solubilizing agents.

[0052] Those skilled in the art of photodynamic therapy and compounds related to the presant invention will be bettar able to determine an appropriate dosage and overall dosage regime when taking a number of factors into consideration. For example, the size, weight and condition of the patient must be considered as must be the responsiveness of the patient and their disease to the particular therapy. It is believed that even relatively small doses administered a single time can have a beneficial effect. Further, extremely large doses could, of course, be toxic. Accordingly, rather than providing specific information on dosage amount and intervals between dosing, attention should be paid to conventional factors used in determining such dosing while considering that the pyropheophorbide compounds of the invention have a greater degree of toxicity with respect to tumor cells and therefore can generally be administered in smaller amounts than the convantional compounds usad in connaction with photodynamic therapy.

EXAMPLES

[0053] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosura and description of how to make the pyropheophorbide compounds and pharmaceutical compositions of the invention and ara not intended to limit the scopa of what the invantors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.), but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in degrees Centigrade, and prassura is at or near atmospheric.

EXAMPLE 1

[0054] **Methyl pyropheophorbide-a (2) :** Methyl pheophorbide-a (1, 1.0 g) was obtained from alga Spirulina destridratada by following the procadure described in K.M. Smith, D.A. Goff and D.J. Simpson, J. Am. Chem. Soc., 1985, 107, 4941-4954; and R.K. Pandey, D.A. Bellnier, K.M. Smith and T.J. Dougherty, Photochem. Photobiol., 1991, 53, 65-72, both of which ara incorporated herein by reference. The methyl pheophorbide-a was heated under reflux in collidina (100 ml) for 90 min during slow passaga of a stream of nitrogan. Sea G.W. Kenner, S.W. McCombia and K. M. Smith, J. Chem. Soc. Perkin Trans. 1973, 1, 2517-2523, incorporated herein by reference. The solution is avaporated (0.1 mm Hg) and the residue was recrystallized from dichloromethane/methanol. Yield 820 mg; 91%, m.p. 217-219°C, lit. 220-225°C; H. Fishar and A. Stern, Die Chamie des Pyrrole, vol II, Part 2, pp. 64 and 74, Akademische Verlag, Leipzig Incorporated herein by reference. Vis: (max) 410 (112 000); 508 (11 000); 536 (9 600); 610 (4 200); 666 (45 000); NMR, ppm; 9.50, 9.38, 8.52 (each s, 3H, 3 meso H); 7.95-8.05 (m, 1H, CH=CH_2); 6.30 and 6.15 (each s, 1H, CH-CH_2); 5.27 to 5.12 (q, 2H, 10-CH_2); 4.50 (m, 8-HO, 4.28 (m, 7-H); 3.70 (q, 2H, CH_2CH_3); 3.68 (s, 3H, $CHCHCO_2CH_3$); 3.62, 3.40, 3.22 (each s, 3H, $3CH_3$); 2.70 (7a-H); 2.31(7a'-H); 2.56 (7b-H); 2.29 (7b'-H); 1.82 (d, 3H, 8-CH_3); 1.70 (t, 3H, CH_2CH_3); 1.70 (s, 2H,2 NH)

[0055] **Pyropheophorbide-a (3):** Methyl pyropheophorbide-a (2, 250 mg) was dissolved in distilled tetrahydrofuran (50 ml) and 4N HCl (125 ml) was added in one lot. The reaction mixture was stirred under nitrogen atmosphere at room temperature for 4 hours. The reaction was monitored by analytical tlc (silica plates), using 10% methanol/dichloromethane as a mobile phase. The reaction mixture was then poured in ice water, extracted with dichloromethane. The dichloromethane layer was washed several times with water (3X200 ml). The organic layer was separated and dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was crystallized from dichloromethane/hexane. Yield, 225 mg. The purity of the compound was ascertained by tlc and the structure was confirmed by NMR spectroscopy. The NMR data were similar as described for 2 except the resonances for the -OCH_3 protons of the propionic ester (-$CH_2CH_2CO_2CH_3$) were missing.

[0056] **Methyl -2 - (1(O-hexyl)ethyl) - devinyl pyropheophorbide (4):** Pyropheophorbide-a (2, 200 mg) was dissolved in 30% HBr/acetic acid (5.0. ml) and the reaction mixture was stirred in a glass stoppered flask (rubber septum

can also be used) at room temperature for 2.5 hours. The solvent was removed under high vacuum (1 mm Hg) and the resulting 1-bromo ethyl derivative was immediately treated with n-hexanol (3.0 ml) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 45 min, diluted with dichloromethane (100 ml). The dichloromethane layer was washed with water (3X200 ml) till the aqueous phase is neutral and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed over Alumina Grade III (6% water/neutral Alumina) and eluted with dichloromethane. The first fraction was a mixture of the starting material and the desired product (minor quantity). Further elution with same solvent gave the desired product. The appropriate eluates were combined. Evaporation of the solvent afforded a sticky solid, which can be crystallized from dichloromethane/ hexane. Yield 70%. (see Scheme-1), Vis (max): 408 (90 000); 471 (9 200), 505 (8600); 536 (8,500); 604 (7,250); 660 (41 500). NMR, ppm: 9.79, 9.51, 8.53 (each s, 1H, meso H); 5.90 (q, 2H, -CH (O-hexyl)CH_3; 5.08-5.30 (q, 2H, 10 - CH_2); 4.47 (m, 8H); 4.29 (m, 7-HO; 3.75 (q, 2H, CH_2CH_3); 3.67 (s, 3H, $CH_2CH_2CO_2CH_3$), 3.67 (s, 6H, 2 X CH_3); 3.38 and 3.27 (each s, CH_3); 2.68 (7a-H) 2.28 (7a'-H), 2.55 (7b-H); 2.20 (7b'-H); 1.80 (d, 3H, CH_2CH_3); -1.70 (s, 2H, 2 NH); for the hexyl group, 3.72 (t, 2H, O-CH_2CH_2); 1.73 (2H, CH_2); 1.25 [bs, merged, 6H, $(CH_2)_3$]; 0.78 (t, 3H, CH_3). (see fig. 1).

[0057] 2-(1(O-hexyl)ethyl)devinyl pyrophaophorbide-a (5): Pyropheophorbide-a (3, 200 mg) was reacted with 30% HBr/acetic acid and then with n-hexanol by following the method as discussed for 4 and the desired product was isolated in 60 to 65% yield. The structure was confirmed by NMR spectroscopy.

EXAMPLE 2

Tumor Treatment

[0058] When 2-[1-(O-hexyl)ethyl] devinyl pyropheophorbide-a - structure (5) in Scheme 1: S-RO- where R = $(CH_2)_5CH_3$ and m = H, (formula IIa) synthesized as indicated (5.0 mg) is dissolved in Tween 80 (0.1 ml) and mixed with 10 ml Hanks Balanced Salt Solution (HBSS), a solution of approximately 0.5 mg/ml in 0.1% Tween 80 is produced after filtration through a 0.22 µM Millipore filter. Ten DBA/2 mice with 0.4-0.5 mm diameter subcutaneous SMT-F tumors in the axilla are injected intravenously with 0.3 mg/kg body weight of the above solution (after diluting in HBSS so that the injected volume per mouse is approximately 0.2 ml). Approximately 24 h later the tumor area (having been shaved and depilated prior to tumor implant) is exposed to laser light at 660-670 nm for 30 min at a power of 75 mW/cm^2 to deliver 135 Joules/cm^2. Alternately, a Xenon arc lamp filtered to emit a broader band width near 670 nm and approximately 283 Joules/cm^2 can be used.

[0059] The day after light treatment all the tumors are seen to be flat (non-palpable) and slight skin blanching over the area is noted. This progresses to frank tumor necrosis over the next few days. At 7 days post treatment all tumors remain non-palpable and necrotic. At 30 days post treatment, six of the 10 tumors remain non-palpable, and one remains tumor-free to 90 days post treatment.

EXAMPLE 3

Skin Clearance

[0060] Six albino Swiss mice (HsICR) are injected intravenously with a dose of 0.1 mg/kg body weight of the compound of formula IIa prepared as in Example 1. After approximately 24 h, the hind foot of the animal is exposed to the same dose of either lasar light at 660-670 nm (135 Joules/cm^2) or the Xenon arc lamp (283 Joules/cm^2) as above. The reaction of the foot is scored for damage over the next few days to determine the maximum effect, which in this case is a value 0.3 equivalent to slight edema. If the internal between the injection and light treatment is extended to approximately 48 h, the foot reaction is zero (no damage incurred), indicating either clearance or metabolism of the sensitizer.

[0061] Data obtained as a result of experiment carried out is put forth below in Table 1.

In Vivo Tumor Photosensitizing Activity of Pyropheophorbide Ethers[1]

Injected Dose (mg/kg) Formula II R_1 = -O-CH(CH$_3$)CH$_3$ R_2 = -CH$_2$H	Time Interval (hours)	Wavelength	Tumor Response[2]				Normal Foot Response[3]		Maximum Reaction
			Day 1[4]	Day 3	Day 10	Day 90	Time Interval (hours)		
0.05	24	659	0/40	-	-	-			
0.1	24	659	4/4	4/4	1/4	1/4	24		0.3
0.3	24	659	5/5	4/5	0/5	-	48-72		0
0.3	24	655	0/10	-	-	-	-		-
0.3	24	665	10/10	10/10	2/10	0/10	-		-
0.3	24	670	10/10	10/10	6/10	1/10	-		-
0.3	24	680	0/10	0/10	-	-	-		-
Formula II R_1 = -O-CH(CH$_3$)CH$_3$ R_2 = -CO$_2$CH$_3$									
0.3	24	660	4/4	4/4	3/4	3/4	-		-
0.1	24	660	5/5	3/5	0/5	-	-		-
Formula II R_1 = -O-CH$_3$ R_2 = -CO$_2$CH$_3$									
0.1	24	660	0/6	-	-	-	-		-
0.5	3	660	0/4	-	-	-	-		-
Formula I R_1 = -CH(OCH$_3$)CH$_3$ R_2 = -CO$_2$CH$_3$									
0.3	24	660	4/4	2/4	0/4	-	-		-

[1]SMT-F tumor in DBA/2 mice; 135 J/cm^2 light from laser at 75 mW/cm^2

[2]Number of non-palpable tumors/Number treated tumors post light treatment on Day indicated

[3]White Albino Swiss mice; foot exposed using same conditions as for tumor treatment. Score of 0.3 = light edema, 0 = no reaction

[0062] The data put forth in Table 1 clearly demonstrates that the pyropheophorbide compounds of the invention are activated by light having a wavelength of about 660 nm. Further, when the compound were administered by injection and subjected to light having a wavelength of about 660 nm, the treatment was found to be highly effective with respect to reducing tumor size in as little as seven days.

[0063] Further, the data of Table 1 show compounds of the invention clear skin over a period of 24-48 hours after administration. This is a desirable feature in that the patient is not subjected to prolonged cutaneous photosensitivity.

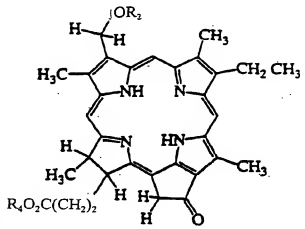
The data of Table 1 also show that the hexyl ethers of formula II are preferred over methyl ethers in terms of effecting tumor growth when used in photodynamic therapy.

[0064] While the present invention has been described with reference to specific compounds, formulations and methods, it is to be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt to a particular individual, method of administration, process of synthesizing, etc., which are within the scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

Claims

1. A compound of formula I:

I



wherein R_2 is a primary or secondary alkyl containing 1 to 20 carbons; and R_4 is H or an alkyl containing 1 to 20 carbons.

2. The compound as claimed in claim 1, wherein R_2 is hexyl; and R_4 is CH_3.

3. Use of a compound of formula I as defined in claim 1 in the manufacture of a medicament for use in the destruction of target virus, cells or tissue.

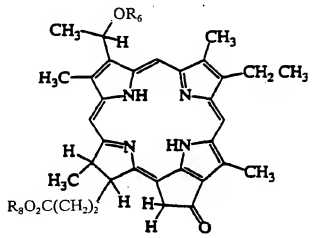
4. A pharmaceutical composition useful in treatment of a target virus, cells or tissue, comprising:
   an effective amount of the compound of claim 1 in admixture with a pharmaceutically acceptable excipient.

5. A conjugate which consists essentially of the compound of claim 1 covalently bound to a target specific component selected from the group consisting of an immunoglobulin and a receptor ligand.

6. A pharmaceutical composition useful for labelling malignant tissue which comprises the compound of claim 1 associated with a label.

7. A compound of formula II:

II



wherein R_6 is a primary or secondary alkyl containing 1 to 20 carbons and R_8 is H or an alkyl containing 1 to 20 carbons.

8. The compound as claimed in claim 7, wherein R_8 is selected from the group consisting of hexyl and $(CH_2)_5CH_3$ and R_8 is selected from the group consisting of CH_3 and H.

9. Use of a compound of formula II as defined in claim 7 in the manufacture of a medicament for use in the destruction of target virus, cells or tissue.

10. A pharmaceutical composition useful in treatment of a target virus, cells, or tissue, comprising:
an effective amount of the compound of claim 7 in admixture with a pharmaceutically acceptable excipient.

11. A conjugate which consists essentially of the compound of claim 7 covalently bound to a target-specific component selected from the group consisting of an immunoglobulin and a receptor ligand.

12. Use of a compound of formula II as defined claim 7 in the manufacture of a medicament for use in a method of treating a human with abnormal cells which replicate at an abnormally high rate.

13. Use of a compound of formula I as defined in claim 1 in the manufacture of a medicament for use in a method of treating a human with abnormal cells which replicate at an abnormally high rate.

14. Use according to claim 12, wherein R_6 is hexyl and R_8 is H and wherein the wavelength of the light is about 660 nm.

FIG. 1